

Appl. No. : 10/764,978
Confirmation No. : 9303
Applicant : Plamen Denchev

Filed : January 23, 2004
Title : METHODS FOR REPRODUCING
CONIFERS BY SOMATIC
EMBRYOGENESIS

IC/A.U. : 1661
Examiner : Hwu, June

Docket No. : 205502-9037-US00

I, Sally Sorensen, hereby certify that this correspondence is being electronically filed with the United States Patent and Trademark Office on the date of my signature.

Sally Sorensen
Signature
May 16, 2008
Date of Signature

**DECLARATION OF STEPHEN ATTREE
UNDER 37 C.F.R. § 1.132**

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Stephen Attree, do hereby declare and state the following:

1. I am currently a Director of Intellectual Property at CellFor, Inc. ("CellFor"). I have been employed by CellFor since January 1, 2000.
2. I received a Bachelors of Science Degree in Botany from the University of Manchester, UK in 1982. I received a Ph.D. in Plant Biology from the University of Manchester in 1987.
3. Attached hereto as Exhibit A is a list of my relevant patents and publications.
4. I am a co-inventor of the subject matter of all claims pending in the above-identified patent application. I make this declaration in support of prosecution of the subject application before the U.S. Patent and Trademark Office ("USPTO").
5. I have read and understand the invention as disclosed in the above-identified patent application, including the invention described by the presently pending claims.

6. I have reviewed the Office Action of January 16, 2008. I understand that claims 1, 5-9, 12-13, 16-23, 27, 28, 33-34, and 36-43 are rejected under 35 U.S.C. § 103(a) as unpatentable over Attree (U.S. Patent No. 6,627,441) in view of Handley (U.S. Patent No. 5,491,090). I also understand that claims 50-54 are rejected under 35 U.S.C. § 103(a) as unpatentable over Fan (U.S. Patent No. 6,689,609) in view of Handley. I also understand that claims 55-60 are rejected under 35 U.S.C. § 103(a) as unpatentable over Coke (U.S. Patent No. 5,534,433) in view of Pullman (U.S. Patent No. 6,492,174). I believe that the evidence presented herein demonstrates that the pending claims are not obvious in light of the cited references.
7. The pending claims are drawn to methods for reproducing coniferous somatic embryos by somatic embryogenesis comprising growing an embryogenic culture derived from an explant on a nutrient medium comprising lactose, lactose and an additional sugar or a galactose-containing sugar and an additional sugar in steps prior to the maturation step, namely the induction, maintenance and/or prematuration steps.
8. Induction, maintenance and prematuration are steps prior to maturation and the media used during these steps help the conifer cells to remain undifferentiated and to proliferate. The media used during induction, maintenance and prematuration generally contain a metabolizable carbon source, hormones such as auxin and/or cytokinin and have a low osmoticum. Maturation requires the cells to slow or stop proliferating and differentiate. Maturation media generally have no auxin or cytokinin, have ABA added and have a relatively high osmoticum. Germination requires further differentiation to form seedlings. Germination media also do not contain auxin or cytokinin and generally have a low osmoticum. Induction, maintenance and prematuration require the cells to proliferate and remain in an undifferentiated state, whereas maturation and germination require the cells to stop proliferating and differentiate. Because the goals at these different steps of the process are exactly opposite, the media used at different stages of the method are, and would be expected to be, distinct.
9. U.S. Patent No. 6,627,441 to Attree relates to methods of promoting maturation of embryos by increasing the water stress on the embryos during the maturation step. See

abstract. The Examiner suggests that Attree teaches use of lactose in prematuration medium at Table 5 and column 26, lines 25-38. Attree clearly indicates that the media in Table 5 which contain lactose are maturation media and not prematuration media. See column 26, lines 26-30 ("Thus, immature somatic embryos from suspension culture were...transferred to maturation medium containing 3% sucrose, 20 μ M ABA and adjusted to 290mmol/kg with PEG." Emphasis added). This first maturation medium represents week 1 in Table 5 and the medium was replaced weekly during maturation with the media indicated in Table 5. Thus, lactose was first added in the third week of maturation, not during prematuration as indicated by the Examiner.

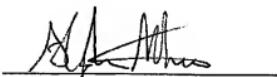
10. In addition, Attree makes clear that the lactose in the maturation media was used as an osmoticum to increase the water stress. See column 26, lines 34-35 ("water potential was increased by adding lactose"). Thus, Attree was using lactose, not as a carbon source, but instead as a means of increasing the water stress on the cells. During maturation the increased water stress reduces the moisture content of the embryos, enhances lipid storage and enhances development into mature embryos capable of germination. These effects, while being important for maturation of the embryo, are the opposite of the desired effects during induction, maintenance and prematuration of the embryos. In induction, maintenance and prematuration, a low water stress (osmoticum) is desired. Thus, the disclosure of Attree that lactose could be used as an osmoticum in the maturation medium actually discouraged the use of lactose in the induction, maintenance or prematuration stages of somatic embryogenesis.
11. Prior to the results presented in the present application, lactose, a sugar found in milk and not generally available to plants, was not believed to be metabolized by plants. The fact that lactose could be used as a carbon source was unexpected as noted in the specification at least at page 6, lines 6-9, in Example 5, page 13-14 and Example 5.1, page 14. Without the knowledge that lactose could be used as a carbon source, there would be no reason to add lactose to the induction, maintenance or prematuration media.
12. In addition, before actually doing the experiments, we would not have predicted that use of lactose in the media during induction, maintenance and/or prematuration would have

such a beneficial effect in terms of producing somatic embryos as compared to sucrose or maltose. For example, Example 1 shows over 4 fold better induction for loblolly pine in a combination of lactose and glucose than in sucrose. Example 3 demonstrates about a 2 fold increase in somatic embryos per gram of tissue when lactose was used as the sole carbon source during maintenance of loblolly pine cultures. Similar results were obtained with Radiata Pine (Examples 6 and 7).

13. The superior results obtained using these methods could not have been predicted. Even if one would have thought that lactose or galactose would be effective carbon sources for use in induction, maintenance and prematuration, the results demonstrating much higher numbers of somatic embryos per gram of tissue were surprising. This represents a significant improvement in the field because maintenance and bulk-up of tissues is a large expense and by generating higher numbers of embryos per gram of tissue the costs of somatic embryogenesis can be decreased significantly. The unexpected benefits of using a galactose-containing sugar as compared to other more traditionally used sugars were noted in the specification at least at page 6, lines 23-25 and page 8, lines 15-21. These unexpected benefits seem to be generic to conifers as all three conifers tested demonstrated a significant improvement in the number of somatic embryos produced per gram of tissue when a galactose-containing sugar was used in induction, maintenance and/or prematuration media.
14. For the reasons set forth above in paragraphs 8-13, the results demonstrated in the Examples section of the present application are surprising and would not be expected based on the cited references.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements and the like so made are punishable by fine or imprisonment or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: 13 May 2008



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Exhibit A

Relevant Patents and Publications: Stephen Attree, Ph.D.

Issued U.S. Patents

Attree, S.M. (2003). Increasing levels of growth regulator and/or water stressing during embryo development. United States Patent 6,627,441

Attree, S.M., Fowke, L.C. (2002). Desiccation tolerant Gymnosperm embryos. United States Patent 6,372,496

Attree, S.M., Fowke, L.C. (2002). Production of desiccation tolerant Gymnosperm embryos. United States Patent 6,340,581.

Attree, S.M., Fowke, L.C. (1999). Maturation, desiccation and encapsulation of gymnosperm somatic embryos. United States Patent 5,985,667.

Attree, S.M., Fowke, L.C. (1995). Desiccated conifer somatic embryos. United States Patent 5,464,769.

Patent applications

Ilic-Grubor, K., Attree, S.M., Fowke, L.C. Media and Methods for culturing plant embryos. PCT filed 1998.

Denchev, Attree, Kong, Tsai, Radley, Lobatcheval. A method for producing conifers by somatic embryogenesis using galactose containing compounds as a carbon and energy source. Filed 2003.

Lobatcheva, Attree, Liu and Williams. Bulk sorting of conifer somatic embryos. Filed 2003.

Rise, Grossnickle, Fan, Attree, Denchev, Krol, Shang. Aerated liquid priming of loblolly pine somatic embryos. Filed 2003.

Fan, Grossnickle, Rise, Attree, Folk. Method of ex vitro sowing, germination, growth and conversion of plant somatic embryos or germinants, and nutrient medium used therefore. Filed 2003

Kong, Denchev, Lobatcheva, Attree, Radley. Method of culturing conifer somatic embryos using S(+) abscisic acid. Filed 2005.

Refereed Journal Contributions

Research papers

- Reid, D.A., Lott, J.N.A., Attree, S.M., Fowke, L.C. (1999) Imbibition of white spruce seeds and somatic embryos: A study of morphological changes in an environmental scanning electron microscope and potassium leakage. In Vitro Cellular and Developmental Biology-Plant 35:303-308.
- Reid, D.A., Lott, J.N.A., Attree, S.M., Fowke, L.C. (1999) Mineral nutrition in white spruce (*Picea glauca*) [Moench] Voss seeds and somatic embryos. I. Phosphorus, phytic acid, potassium, magnesium, calcium, iron, and zinc. Plant Science 141:11-18.
- Reid, D.A., Lott, J.N.A., Attree, S.M., Fowke, L.C. (1999) Mineral nutrition in white spruce (*Picea glauca*) [Moench] Voss seeds and somatic embryos. II. EDX analysis of globoids and Fe-rich particles. Plant Science 141:19-27.
- Kong, L., Attree, S.M., Fowke, L.C. (1998). Effects of polyethylene glycol and methylglyoxal bis(guanylhydrazone) on endogenous polyamine levels and somatic embryo maturation in white spruce (*Picea glauca*). Plant Science 133:211-220.
- Ilic-Grubor, K., Attree, S.M., Fowke, L.C. (1998). Comparative morphological study of zygotic and microspore-derived embryos of *Brassica napus* L. as revealed by scanning electron microscopy. Journal of Experimental Botany 82:157-165.
- Ilic-Grubor, K., Attree, S.M., Fowke, L.C. (1998). Induction of microspore-derived embryos of *Brassica napus* L. with polyethylene glycol (PEG) as osmoticum in low sucrose medium. Plant Cell Reports 17:329-333.
- Evans, D.E., Clay, P. J., Attree, S.M., Fowke, L.C. (1997). Visualization of Golgi apparatus in methacrylate embedded conifer embryo tissue using the monoclonal antibody JIM 84. Cell Biology International 21: 295-302
- Kong, L., Attree, S.M., Fowke, L.C. (1997). Changes of endogenous hormone levels in developing seeds, zygotic embryos and megagametophytes in *Picea glauca*. Physiologia Plantarum 101: 23-30.
- Attree, S.M., Pomeroy, M.K., Fowke, L.C. (1995). Development of white spruce (*Picea glauca* [Moench.] Voss) somatic embryos during culture with abscisic acid and osmoticum, and their tolerance to drying and frozen storage. Journal of Experimental Botany 46: 433-439.
- Leal, I., Misra, S., Attree, S.M., Fowke, L.C. (1995). Effect of abscisic acid, osmoticum and desiccation on 11S storage protein gene expression in somatic embryos of white spruce. Plant Science 106: 121-128.

- Attrie, S.M., Pomeroy, M.K., Fowke, L.C. (1994). Production of vigorous desiccation tolerant white spruce (*Picea glauca* [Moench.] Voss) synthetic seeds in a bioreactor. *Plant Cell Reports* 13:601-606.
- Fowke, L.C., Attree, S.M., Rennie, P.J. (1994). Scanning electron microscopy of hydrated and desiccated mature somatic embryos and zygotic embryos of white spruce (*Picea glauca* [Moench.] Voss). *Plant Cell Reports* 13:612-618.
- Misra, S., Attree, S.M., Leal, I., Fowke, L.C. (1993). Effect of abscisic acid, osmoticum, and desiccation on synthesis of crystalloid proteins during the development of white spruce somatic embryos. *Annals of Botany* 71:11-22.
- Attrie, S.M., Pomeroy, M.K., Fowke, L.C. (1992). Manipulation of conditions for the culture of somatic embryos of white spruce for improved triacylglycerol biosynthesis and desiccation tolerance. *Planta* 187:395-404.
- Attrie, S.M., Moore, D., Sawhney, V.K., Fowke, L. C. (1991). Enhanced maturation and desiccation tolerance of white spruce (*Picea glauca* [Moench.] Voss) somatic embryos: Effects of a non-plasmolysing water stress and abscisic acid. *Annals of Botany* 68:519-525.
- Attrie, S.M., Tautorus, T.E., Dunstan, D.I., Fowke, L.C. (1990). Somatic embryo maturation, germination, and soil establishment of plants of black and white spruce (*Picea glauca* and *Picea mariana*). *Canadian Journal of Botany*. 68:2583-2589.
- Fowke, L.C., Attree, S.M., Wang, H., Dunstan, D.I. (1990). Microtubule organization and cell division in embryogenic protoplast cultures of white spruce (*Picea glauca*). *Protoplasma* 158:86-94.
- Attrie, S.M., Budimir, S., Fowke, L.C. (1990). Somatic embryogenesis and plantlet regeneration from cultured shoots and cotyledons of seedlings from stored seed of black and white spruce (*Picea mariana* and *Picea glauca*). *Canadian Journal Botany* 68:30-34.
- Tautorus, T.E., Attree, S.M., Fowke, L.C., Dunstan, D.I. (1990). Somatic embryogenesis from immature and mature zygotic embryos, and embryo regeneration from protoplasts of black spruce (*Picea mariana* Mill.). *Plant Science* 67:115-124.
- Attrie, S.M., Dunstan, D.I., Fowke, L.C. (1989). Plantlet regeneration from embryogenic protoplasts of white spruce (*Picea glauca*). *Bio/Technology* 7:1060-1062.
- Attrie, S.M., Dunstan, D.I., Fowke, L.C. (1989). Initiation of embryogenic callus and suspension cultures, and improved embryo regeneration from protoplasts, of white spruce (*Picea glauca*). *Canadian Journal of Botany*. 67:1790-1795.
- Attrie, S.M., Bekkaoui, F., Dunstan, D.I., Fowke, L.C. (1987). Regeneration of protoplasts from an embryogenic suspension culture of white spruce (*Picea glauca*). *Plant Cell Reports* 6:480-483.

- Bekkaoui, F., Saxena, P.K., Attree, S.M., Fowke, L.C., Dunstan, D.I. (1987). The isolation and culture of protoplasts from an embryogenic suspension culture of *Picea glauca* (Moench) Voss. Plant Cell Reports 5:476-479.
- Attree, S.M., Sheffield, E. (1986). An evaluation of ficoll density gradient centrifugation as a method of eliminating microbial contamination and purifying plant protoplasts. Plant Cell Reports 5:288-291.
- Attree, S.M., Sheffield, E. (1985). Plasmolysis of *Pteridium* protoplasts: A study using light and scanning electron microscopy. Planta 165:151-157.
- Attree, S.M., Sheffield, E. (1984). Scanning electron microscopy of protoplasts isolated from gametophytes of the fern *Pteridium*. I. Preparative methods. Micron and Microscopica Acta 15:181-186.
- Sheffield, E., Attree, S.M. (1983). Further experiments with ferns in culture: Regeneration. Journal of Biological Education 17:183-184.

Invited reviews

- Fowke, L.C., Attree, S.M. (1996). Conifer somatic embryogenesis: studies of embryo development and the cell biology of conifer cells and protoplasts. Plant Tissue Culture and Biotechnology 2:124-130.
- Fowke, L.C., Attree, S.M., Binarova, P., Galway, M.E., Wang, H. (1995). Conifer somatic embryogenesis for studies of plant cell biology. In Vitro Cellular and Developmental Biology 31: 1-7.
- Attree, S.M., Fowke, L.C. (1993). Embryogeny of gymnosperms: advances in synthetic seed technology of conifers. Plant Cell Tissue Organ Culture 35:1-35.
- Fowke, L.C., Attree, S.M. (1993). Applications of embryogenic spruce cultures for applied and fundamental research. Biotechnology and Biotechnological Equipment 7: 15-19.

Other Refereed Contributions

Refereed book chapters

- Cyr, D.R., Attree, S.M., El-Kassaby, Y.E., Ellis, D.E., Polonenko, D.R., Sutton, B.C.S. (2001). Application of somatic embryogenesis to tree improvement in conifers. In: Morohoshi, N., Komamine, A., Molecular breeding of woody plants. Progress in Biotechnology breeding. Elsevier, Amsterdam, pp 305-312

- Kong, L., Attree, S.M., Evans, D.E., Binarova, P., Yeung, E.C., Fowke, L.C (1999). Somatic embryogenesis in white spruce for studies of embryo development and cell biology. In: Jain, S. M., Gupta, P.K., Somatic embryogenesis in woody plants Vol III, Kluwer, Dordrecht, pp 1-28.
- Attree, S.M., Rennie, P.J., Fowke, L.C. (1996). Induction of somatic embryogenesis in conifers. In: Trigiano, R.N., Gray, D.J. (eds), Tissue culture of vascular plants: Concepts and laboratory exercises, CRC Press Inc., Boca Raton, pp 191-198.
- Attree, S.M., Fowke, L.C. (1995). Conifer somatic embryogenesis, embryo development, maturation drying and plant formation. In: Gamborg, O.L., Phillips, G.C. (eds), Plant cell tissue and organ culture: fundamental methods, Springer-Verlag, Berlin, pp 103-113.
- Attree, S.M., Fowke, L.C. (1991). Micropropagation through somatic embryogenesis in conifers. In: Bajaj, Y.P.S. (ed.), Biotechnology in agriculture and forestry, High-Tech and Micropropagation, vol 17, Springer-Verlag, Berlin, pp 53-70.
- Attree, S.M., Dunstan, D.I., Fowke, L.C. (1991). White spruce [*Picea glauca* (Moench.) Voss and black spruce [*Picea mariana* (Mill.) B.S.P. In: Bajaj, Y.P.S. (ed.), Biotechnology in agriculture and forestry, Trees III, vol 16, Springer-Verlag, Berlin, pp 423-445.
- Attree, S.M., Sheffield, E. (1986). Protoplasts of *Pteridium* gametophytes. In: Bracken: Ecology, land use and control technology, Smith, R.T. and Taylor, J.A. (eds). Parthenon Publishing, Camforth, Lancs, pp. 315-321.
- Conference proceedings
- Sutton, B, Attree, S.M., El-Kassaby, Y., Cyr, D. (2000). Clonal propagation and tree improvement using somatic embryogenesis. TAPPI Journal .
- Fowke, L.C., Attree, S.M. (1993). Applied and basic studies of somatic embryogenesis in white spruce (*Picea glauca*) and black spruce (*Picea mariana*). In; Woong Young Soh et al. (eds.), Advances in Developmental Biology and Biotechnology of Higher Plants. Korean Society of Plant Tissue Culture, Korea, pp. 5-17.
- Fowke, L.C., Attree S.M (1993). Applications of embryogenic spruce cultures for applied and fundamental research. Biotechnology and Biotechnological Equipment. 7; 15-19.
- Attree, S.M., Bekkaoui, F., Saxena, P.K., Dunstan, D.I., Fowke, L.C. (1988). Differences in developmental morphology of protoplasts from two cell lines of white spruce (*Picea glauca*). In; Puite et al. (eds.), Progress in plant protoplast research. Kluwer Academic Publishers, Dordrecht, pp. 121-122.

Attrie, S.M., Sheffield, E. (1985). Isolation and regeneration of *Pteridium* protoplasts. In: Biology of Pteridophytes, Dyer, A.F. and Page, C.N. (eds.), Proc. Roy. Soc. Edin. The Royal Society of Edinburgh, Edinburgh, pp. 459-460.

Oral presentations

Attrie, S.M. (2005). Developing a Commercial Somatic Embryogenesis Platform for Conifers. Invited presentation, SIVB, Baltimore, Maryland, USA

Attrie, S.M. (2004). Developing a Commercial Somatic Embryogenesis Platform for Conifers. Invited presentation. IUFRO Meeting on Forestry, Charleston, South Carolina, USA

Attrie, S.M. (2001). Applications of micropropagation. Keynote presentation IAPTC June 2001, Saskatoon, Saskatchewan, Canada

Attrie, S.M., Denchev, P., Kong, L., (2000). Clonal propagation of conifers by somatic embryogenesis. 21st annual Forest Vegetation Management Conference, January 18-20 2000, Redding, California.

Attrie, S.M., Kong, L., Denchev., P., Ilic-Grubor, K., Fowke, L.C. (1999). Water stress ABA and desiccation treatments promote somatic embryogenesis in conifers and angiosperms. Congress on in vitro biology, June 5-9,1999, New Orleans. Abstracted in In Vitro 35; W2.

Attrie, S.M., Kong, L., Fowke, L.C. (1996). Recent advances in conifer somatic embryogenesis, invited presentation at the 4th Canadian Plant Tissue Culture and Genetic Engineering Conference, 1-4 June, Saskatoon, Saskatchewan, Canada.

Attrie, S.M., Fowke L.C. (1994). Conifer Synthetic Seeds. Invited presentation at the Second International Symposium on the Application of Biotechnology to Tree Culture, Protection, and Utilization, 2-6 October, Minneapolis, Minnesota, USA.

Attrie, S.M., Fowke, L.C. (1992). Artificial seeds of conifers. 1992 World Congress on Cell and Tissue Culture, June 20-25, Washington, DC, USA. Abstracted in In Vitro 28; 100A.

Attrie, S.M., Fowke, L.C. (1992). Maturation and desiccation of white spruce somatic embryos. IAPTC Canada Section, June 17-19, Guelph, Ontario, Canada.

Attrie, S.M., Fowke, L.C. (1990). Somatic embryo maturation, germination, and soil establishment of plants of black and white spruce (*Picea glauca* and *Picea mariana*). 5th meeting, Conifer Biotechnology Working Group, Shell Research Ltd, Sittingbourne, UK.

Attrie, S.M. Fowke, L.C. (1989). Plant regeneration from protoplasts of *Picea glauca*. Fortieth annual meeting of the Tissue Culture Association, June 11-14, 1989. Orlando, Florida, USA. Abstracted in In Vitro 25:31A.

- Attree, S.M., Dunstan, D.I., Fowke, L.C. (1988). Improved embryo regeneration from protoplasts isolated from ten embryogenic suspension cultures of white spruce (*Picea glauca*). 4th International Conifer Biotechnology Working Group, Saskatoon, Saskatchewan, Canada.
- Attree, S.M., Sheffield, E. (1985). Protoplasts from *Pteridium* gametophytes. Bracken 85, Leeds, U.K.
- Attree, S.M., Sheffield, E. (1983). Scanning electron microscopy of fern protoplasts. Society of Experimental Biology, Exeter, U.K.
- Poster presentations
- Ilic-Grubor, K., Attree, S.M., Fowke, L.C. (1997). Comparative morphological and histological study of zygotic and microspore/pollen-induced embryos of *Brassica napus*. 1997 Congress on In Vitro Biology, June 14-18, Washington DC. Abstracted in In Vitro 33.
- Kong, L., Attree, S.M., Fowke, L.C. (1997). Effects of polyethylene glycol on endogenous polyamine levels during white spruce somatic embryo maturation. 1997 Congress on In Vitro Biology, June 14-18, Washington DC. Abstracted in In Vitro 33.
- Kong, L., Attree, S.M., Fowke, L.C. (1996). Changes of endogenous hormone levels during seed and embryo development in *Picea glauca* (Moench) Voss. 4th Canadian Plant Tissue Culture and Genetic Engineering Conference, 1-4 June, Saskatoon, Saskatchewan, Canada.
- Attree, S.M., Fowke, L.C. (1993) Synthetic seeds of white spruce. 1993 Annual Meeting of the Forest Nursery Association of British Columbia, Sept 13-15th, Courtenay, B.C., Canada.
- Attree, S.M., Fowke, L.C. (1993) Scaling up production of desiccation tolerant conifer somatic embryos. 1993 World Congress on Cell and Tissue Culture, 5th-9th June, San Diego, USA. Abstracted in In Vitro 29P.
- Pomeroy, M.K., Attree, S.M., Fowke, L.C. (1993) Culture conditions for enhanced oil biosynthesis and desiccation tolerance in somatic embryos of white spruce. 1993 Plant Lipid Symposium, July 29-31, Minneapolis, Minnesota, USA.
- Attree, S.M., Fowke, L.C. (1993). Somatic embryogenesis and synthetic seeds of white spruce (*Picea glauca*). XV International Botanical Congress August 28th-September 3rd 1993, Tokyo, Japan.
- Attree, S.M., Fowke, L.C. (1990). Somatic embryo maturation, germination, and soil establishment of plants of black and white spruce (*Picea glauca* and *Picea mariana*). VIIth I.A.P.T.C meeting, June 24-29, Amsterdam, The Netherlands.
- Fowke, L.C., Attree, S.M., Wang, H., Dunstan, D.I. (1990). Microtubule organization and cell division in embryogenic protoplast cultures of white spruce (*Picea glauca*). VIIth I.A.P.T.C meeting, June 24-29, Amsterdam, The Netherlands.

Also shown at 5th meeting, Conifer Biotechnology Working Group, Shell Research Ltd, Sittingbourne, UK.

Tautorus, T.E. Attree, S.M., Fowke, L.C., Dunstan, D.I. (1989). Somatic embryogenesis and protoplast culture in Picea mariana Mill. (black spruce). Fortieth annual meeting of the Tissue Culture Association, June 11-14, 1989. Orlando, Florida, USA. Abstracted in In Vitro 25:63A.

Attree, S.M., Bekkaoui, F., Saxena, P.K., Dunstan, D.I., Fowke, L.C. (1988). Differences in developmental morphology of protoplasts from two cell lines of white spruce (Picea glauca). I.A.P.T.C., Ottawa, Canada.

Attree, S.M., Bekkaoui, F., Saxena, P.K., Fowke, L.C., Dunstan, D.I. (1987). The isolation and culture of protoplasts from an embryogenic suspension culture of Picea glauca. 7th International Protoplast Symposium, 1987, Wageningen, The Netherlands.

Attree, S.M. (1987). Wall regeneration of Pteridium gametophyte protoplasts. Society for Experimental Biology, Manchester, U.K.

Attree, S.M., Sheffield, E. (1983). Isolation and regeneration of Pteridium protoplasts. Biology of Pteridophytes. International symposium, Edinburgh, U.K.

Attree, S.M., (1983). Low temperature SEM of Pteridium aquilinum protoplasts. SEB, Exeter, U.K.

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